

at both X-band and W-band to probe the arm region dynamics. Higher sensitivity to the fast motional dynamics of the spin label at W-band resolved differences in two of the EcoRI complexes that were not apparent in the X-band CW spectra. Molecular dynamics (MD) simulations were performed on the spin-label-modified specific EcoRI-DNA crystal structure to model the average nitroxide orientation. Disparity in average distance as well as distribution indicates a need for further sampling of the spin label in silico. This work is supported by NSF.

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Site-Directed Spin-Labeling Studies: Structure of the AnkD34-Cdb3 Complex

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The adaptor protein ankyrin-R interacts via its membrane binding domain with the cytoplasmic domain of the anion exchange protein (AE1) and via its spectrin binding domain with the spectrin based membrane skeleton in human erythrocytes. This set of interactions provides a bridge between the lipid bilayer and the membrane skeleton thereby stabilizing the membrane. Atomic resolution structures for the dimeric cytoplasmic domain of AE1 (cdb3) and for a twelve ankyrin repeat segment (repeats 13-24) from the membrane binding domain of ankyrin-R (AnkD34) have been reported. However, structural data on how these proteins assemble to form a stable complex have not been reported. In the current studies, site directed spin labeling, in combination with electron paramagnetic resonance (EPR) and double electron-electron resonance (DEER), have been utilized to map the binding interfaces of the two proteins in the complex and to obtain inter-protein distance constraints. These data have been utilized to construct a family of structural models that are consistent with the full range of experimental data. These models indicate that an extensive area on the peripheral domain of cdb3 binds to ankyrin repeats 18-20 on the top convex surface of AnkD34 along the flexible linker away from the ankyrin groove primarily through hydrophobic interactions. This is a previously structurally uncharacterized surface for binding of cdb3 to AnkD34. Since a second dimer of cdb3 is known to bind to ankyrin repeats 7-12 of the membrane binding domain of ankyrin-R, the current models have significant implications regarding the structural nature of a tetrameric form of AE1 that is hypothesized to be involved in binding to Ankyrin-R in the erythrocyte membrane. Supported by NIH P01 GM080513.

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EPR Spectra of Spin-Labeled OmpA Reflect Both Protein Dynamics and Label Configuration in Membrane Mimetic Systems

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Electron paramagnetic resonance (EPR) spectroscopy coupled with site-directed spin labeling (SDSL) is a powerful tool that can provide information on the structure and dynamics of proteins. It has been used successfully in both water soluble and membrane proteins. Because SDSL has no molecular weight limitation, it can be performed under physiological conditions and can give data on systems inaccessible to techniques such as solution NMR and X-ray crystallography. The EPR spectra of labeled sites in the outer membrane protein OmpA are highly dependent upon environment, and generally the spectra indicate that labels (R1 side chain) have less motional averaging in the presence of lipids than in detergent systems. In proteins, EPR spectra are known to be modulated by backbone dynamics. This suggests that OmpA is more dynamic on the ns timescale in detergents than in lipid bilayers. In the present study, continuous wave (cw) and pulse EPR techniques were used to examine the basis for changes in the EPR lineshapes of spin-labeled OmpA. In aqueous exposed regions of the protein, lineshape changes as a function of environment likely reflect changes in protein dynamics; however, within the membrane hydrocarbon region, changes in label dynamics may be influenced by the configuration of the label. The results suggest that as the polarity and/or proticity of the environment decreases, the R1 side chain preferentially interacts with the protein backbone. This result is supported by double electron-electron resonance (DEER) measurements, which can only be accounted for by changes in label rotameric states as a function of environment. The results indicate that in addition to protein dynamics, EPR spectra may be strongly modulated by spin label interactions with the surface of membrane proteins. (Supported by NIGMS GM-035215.)

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Understanding the Membrane Docking Geometry of GRP1 PH Domain Using Site-Directed Spin-Labeling and EPR

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PH domains are membrane targeting motifs that typically recruit signaling proteins to specific membrane surfaces in response to the appearance of lipid second messengers. Despite their functional significance, little is known about how

PH domains dock to the bilayer surface on a structural level. Here we focus on the representative PH domain of the General Receptor for Phosphoinositides 1 (GRP1 PH), which specifically binds the signaling lipid phosphatidylinositol (3,4,5)-triphosphate (PIP3). To elucidate the membrane docking geometry of this PH domain, site-directed spin-labeling and EPR power saturation method are being employed. Single cysteine residues are introduced at positions scattered over the membrane binding face and subsequently labeled with a thio-reactive spin-label. The EPR power saturation method is utilized to measure the bilayer depth of each spin-labeled side chain when the PH domain is docked to a PIP3-containing synthetic lipid bilayer. The resulting membrane depths, in conjunction with the known crystal structure, will be used to model the penetration depth and angle of the PH domain relative to the bilayer surface. The latest findings will be presented at the meeting.

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Pulsed Electron Spin Resonance Resolves the Coordination Site of Cu²⁺ Ions in 1-Glycine Receptor

Sharon Ruthstein, Katherine M. Stone, Timothy M. Cunningham, Ming Ji, Michael Cascio, Sunil K. Saxena.

In this talk, we will discuss the coordination environment of copper ions (Cu²⁺) in human $\alpha 1$ glycine receptor (GlyR). GlyRs are members of the pentameric ligand-gated ion channel (pLGIC) superfamily that mediate fast signaling at synapses. Metal ions like Zn²⁺ and Cu²⁺ significantly modulate the activity of pLGICs, and in vivo results suggest that metal ion coordination might be essential for proper physiological postsynaptic inhibition by GlyR. In order to better understand the molecular basis of this effect we have used pulsed electron spin resonance (ESR) methods like electron spin echo envelope modulation (ESEEM) and double electron electron resonance (DEER) spectroscopy to directly examine Cu²⁺ coordination with GlyR. We show, that Cu²⁺ has one binding site per $\alpha 1$ subunit, and that five Cu²⁺ can be coordinated per GlyR. Cu²⁺ binds to GlyR with a 40 μ M apparent dissociation constant, and the metal ion are coordinated to E192 and H215 in each subunit. This is consistent with earlier functional measurements. However, the coordination site does not include residues around the agonist/antagonist binding site, such as T112, R131, R65, E157 that were previously suggested to have roles in Cu²⁺ coordination by functional measurements on site-directed mutants. Intriguingly, the E192 and H215 are also known to bind Zn²⁺. The binding of Zn²⁺ at this site potentiates channel activity, whereas Cu²⁺ has an inhibitory effect. The opposing modulatory actions of these similar metal divalent cations at a shared binding site highlight the sensitive allosteric nature of GlyR. This work is supported by NIH (5R01NS053788). S.R. is supported by a Long-term Postdoctoral Fellowship awarded by the EMBO.

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ESR Spectroscopy Suggests Unequal Contributions of the Three Histidine Residues to Cu(II) Binding in Amyloid- β at Physiological PH

Byong-kyu Shin, Sunil K. Saxena.

We provide precise information about the contribution of each of the three histidine residues, His6, His13, and His14, to Cu(II) binding in the equimolar Cu(II)-A β (1-16) complex at physiological pH by employing electron spin resonance spectroscopy. Based on our experimental results, we propose probable Cu(II)-coordination environments for the major and the minor component of the Cu(II)-A β (1-16) complex.

Three-pulse electron spin-echo envelope modulation (ESEEM) experiments conducted on nonlabeled and ¹⁵N-labeled Cu(II)-A β (1-16) complexes at a pH of 7.4 reveal that the contributions of the three residues to the Cu(II) coordination are in the order of His14 \approx His6 > His13 in the major component of the Cu(II)-A β (1-16) complex. Also, the ESEEM spectra of diethylenetriamine (DETA)-Cu(II)-A β (1-16) complexes suggest that the relative Cu(II)-binding affinities of the three histidine residues are in essentially the same order as in the major component. The corresponding hyperfine sublevel correlation spectra show that multiple histidine residues coordinate to Cu(II) and suggest the presence of axially bound water in the Cu(II)-coordination environment. In addition, a broader interpretation of our results suggests a significant contribution from the simultaneous coordination of His13 and His14 in the major component, which has been underappreciated, and indicates the possibility of intermolecular bridges through His13 in the minor component.

Our strategy and experimental results are meaningful in that the subtle difference in the three histidine residues at physiological pH is traced by ¹⁵N-labeling and introduction of DETA, a tridentate ligand, without any side chain modification, amino acid residue replacement, or pH change, each of which might lead to an alteration in the peptide structure or the coordination environment.